

DROSOPHILA COURTSHIP: DECAPITATED
QUINARIA GROUP FEMALES

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Abstract.—Grossfield, Joseph, Department of Biology, City College, N.Y., N.Y., 10031.—The behavior of three quinaria group species of *Drosophila*, *D. falleni*, *D. occidentalis* and *D. guttifer* was observed. Males of each species were given a choice of normal and decapitated conspecific females. Males of *D. occidentalis* and *D. guttifer* fail to inseminate any decapitated females while *D. falleni* inseminates significantly fewer decapitated females. Normal females are courted preferentially as evidenced by either the number or the sequence of courtships. Of the three species, *D. guttifer* is the most effective in converting courtship to inseminations with an effective courtship ratio (C/I) of 1.2. Decapitated females do not give acceptance signals. This excludes the possibility that this motor pattern is part of a reflex arc analagous with vertebrates. For some species visual stimuli are involved within the courtship sequence as well as in the initiation of courtship. The recognition by males of these visual signals serves as a gating point for the next step in his courtship sequence. The acceptance response can serve as trigger mechanism for the male motor response. The behavioral architecture of these species differs from the *D. melanogaster* pattern. The discriminatory mechanisms responsible for specific stimulus recognition represent the intrinsic component of behavior least sensitive to environmental modification.

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Introduction

Spieth (1966) used decapitated females to study factors responsible for the initiation of courtship in *Drosophila* and indicated the importance of the female's CNS in interpreting stimuli of male origin. Subsequent studies (Grossfield, 1970a, 1972) reported that males of species which require light in order to mate do not court decapitated females unless the approach is such that the effect of the operation is not evident. This work also suggested that such species use visual cues even after the initiation of courtship. The potential utility of light dependent species for dissection of specific sign stimuli involving a single sensory modality prompts a more detailed look at the behavioral organization of such species compared with more well-known species. Most studies of courtship behavior have used species in the melanogaster, obscura, and willistoni species groups in the subgenus *Sophophora* (Spieth, 1968).

Table 1. Number of females dissected (n) and percent inseminated (%) upon presentation of males of each species with a choice between normal and decapitated females. Flies of each species are 11, 7, or 10 days of age, respectively.

	Insemination				Number of courtships				Courtship ratio a/b
	Normal		Decapitated		χ^{2e}	(a)	(b)	χ^{2d}	
	n	%	n	%		Normal	Decapit		
						♀ ♀	♀ ♀		
<i>D. falleni</i>	50	74.0	50	12.0	24.7	13	5	n.s.	2.6
<i>D. occidentalis</i>	30	80.0	30	0.0	36.7	17	5	n.s.	3.4
<i>D. guttifera</i>	75	92.0	75	0.0	124.1	62	18	11.6	3.4

^c All chi-square values calculated with the Yates correction (Croxtan, 1953) and all are significant at the .001 level.

^d Calculated on the basis of a = b.

Thus, several species from the *quinaria* group of the other major subgenus were chosen. The *quinaria* species represent a clearly definable group which is quite divergent in many aspects of its biology (Patterson & Stone, 1952; Throckmorton, 1962; Spieth, 1952). Spieth (1966) also reported that females of all species he used failed to give acceptance responses. The species he observed included a large sample but did not include certain types of acceptance response. These particular *quinaria* group species were chosen to see whether their females performed similarly. *D. falleni* is a species whose mating is strongly inhibited by darkness (Class II or facultative dark maters, Grossfield, 1971), while *D. guttifera* shows complete inhibition of mating by darkness (Class III or obligatory illumination). The strain of *D. occidentalis* used shows a small degree of mating in darkness (Grossfield, 1966). Since the acceptance response appears to serve as a gating point, or switch in some of these species, it may provide information concerning the neural organization of behavior. The advantage of using such species lies in their possession of distinct recognition signals which allow a discrete qualitative evaluation of unique components, contrasted with quantitative measures which might be expected to show a greater degree of inter-strain variation.

Procedures

Mature virgin females were beheaded two to four hours preceding their introduction, together with normal males, into observation vials. Decapitation was performed under brief, humidified CO₂ anaesthetization. Normal females for each vial were treated identically but not beheaded. The University of Texas collection number is listed for each species pre-

ceding the behavioral description. Each vial had five normal males and an equal number of both normal and decapitated females. Vials were observed under a stereozoom microscope for 1 h following the introduction of both sexes and behavioral details as well as the number of courtships were noted. At the end of 24 h the females were dissected, and the number inseminated determined by microscopic examination of both the ventral receptacle and spermathecae for presence of sperm. All observations and fly stocks were in a $21 \pm 1^\circ\text{C}$ room illuminated for 12 h per day. The observation period was from 1700–1800 h since, under these conditions, these species are more sexually active at this time.

Behavior of Species

(See Table 1)

All basic courtship patterns are taken from Spieth (1952). *D. falleni* is there listed as *D. transversa* (see Wheeler, 1960) for a discussion of this change.

Decapitated females, if they do not decamp at first contact with a courting male, use their legs, midlegs especially, to fend off males. In many cases this fending activity breaks contact of the male's foretarsi with the female's abdomen. As Spieth (1966) reported for the species he used, the behavioral repertoire of decapitated females seems limited to decamping from bothersome stimuli, such as courting males, certain cleaning activities, and general repelling actions with their legs and abdomen.

D. falleni—1062.6

The male, after tapping, positions himself behind the female and vibrates one wing in bursts. He then lunges onto the female and attempts intromission. A receptive female spreads her vaginal plates while an unreceptive one repels by fluttering, kicking, depressing or decamping. Males may circle a nonreceptive female while scissoring one wing.

These males inseminate significantly more normal females. In general, males treat decapitated females in a cursory fashion. A male approaching a decapitated female from the front or side generally ignores the female. Males may position at the rear and vibrate one wing in a few desultory bursts before departing. Only if the approach is made directly behind a decapitated female does a male continue courting and attempt mounting. With such rear approaches, males begin to circle the female, reach the front and walk off. Decapitated females generally remain stationary but for attempted mounting, which constitutes first contact, in which case they decamp.

The proportion of decapitated females inseminated is the same as the

proportion of normal females inseminated in darkness (Crossfield, 1966). This may indicate that a certain minority of the population is less dependent on certain, presumably visual, cues than the rest.

D. occidentalis—2175.3

The male taps, goes to the rear and strokes the dorsal portion of the female's abdomen, simultaneously flicking one wing and licking the ovipositor. A receptive female spreads her wings 90° and spreads her vaginal plates. The male then mounts and inserts. Non-receptive females repel by kicking, fluttering their wings, or elevating their abdomens; occasionally males may attempt mounting non-receptive females.

Initial courtship of females shifts rapidly to courtship of normal females. Males court decapitated females briefly and walk off. Thus, although there is no significant difference in the total number of courtships directed towards the two types of females, the temporal distribution of courtships is significant. Males which court for long periods of time do so at the rear. Even with those males that court for extended periods (over 5 min) no female gives an acceptance response, nor do any males attempt to mount. Few females decamp and most merely engage in fending with the metathoracic legs for intermittent brief periods during the courtships.

D. guttifera—2086.3

The male taps, goes to the rear of the female, approaches with outstretched proboscis and licks the ovipositor. Simultaneously, the male strokes the dorsolateral portion of the female's abdomen with his forelegs. Receptive females spread their wings, elevate their abdomens and spread their vaginal plates. Prior to mounting the male must lick the vaginal plate area. Intromission occurs after mounting. Non-receptive females repel by decamping and kicking. The male's abdomen pulsates strongly prior to attempts at mounting.

Males court normal females more than three times as much as decapitated ones and inseminate the former at a high level while failing to inseminate any decapitated females. Males approaching decapitated females from the front walk over them with no indication of sexual recognition and no courtship activity takes place in front of such females. These females are usually quiescent. Males approaching from the side pause before walking by such females. Males which approach from the rear and court in that position occasionally circle a female and stand in front before completing the circling movement. This only occurs with males which have been courting for long periods prior to circling. If males have not been courting long and perform the circling movement, they walk off upon reaching the front. Males courting these females commonly do so for

periods up to 12 min before ceasing activity. Attempted mounting is rare and in those cases where it does occur the female falls forward and does not decamp. Females are still but for irregular bouts of fending activity occurring even up to 10 min after initiation of male activity. Males courting decapitated females eventually cease and walk off. In no case does a female give an acceptance response.

Decapitated females of these species give no acceptance responses and both *D. guttifera* and *D. occidentalis* differ in their relative immobility, upon contact with a male, from other quinaria group species (Grossfield, 1970a). *D. occidentalis* males do not attempt to mount. In those few cases where *D. guttifera* males attempt to mount they are faced with the inability of the female to maintain posture.

With *D. guttifera* and perhaps the other species it appears that stroking is an autocatalytic activity wherein contact stimuli serve to increase the male excitation and prolong the courtship. This serves to insure that a male, once courting, will continue. The higher level of excitation of males that have been courting is also seen in their completion of the circling movement. This has also been noted by Spieth (1966).

The value of the C/I ratio (number of courtships (C)/number of inseminations (I)) can serve as an index of the efficiency with which courtships are converted to copulations. The most effective courtship would yield $C/I = 1.0$, and higher values would indicate departures in efficiency in the direction $C > I$.

Thus, *D. guttifera* appears to have the most effective courtship with a C/I index of 1.2. *D. occidentalis* and *D. falleni* have indices of 1.4 and 2.85 respectively. Parenthetically, of the three species, *D. guttifera* is the easiest and *D. falleni* the most difficult to maintain under conditions prevailing in the laboratory.

Discussion

Bastock & Manning (1955) suggest that excitatory and inhibitory stimuli given by a female can be pooled to give "effective excitation." Decapitated females clearly have a low value of this component of courtship behavior as evidenced by either the number or the temporal sequence of courtships directed towards them.

Another component, discrimination, implies on the part of both males and females, an ability to distinguish among potential partners. This capability allows females to react by sampling more of a male's courtship or by attempting to discontinue it, and males to react by proceeding with, or breaking off, courtship. High discrimination on the part of females may imply effective repelling of male overtures, but decapitated females, especially *D. occidentalis* and *D. guttifera* show that it is pos-

sible to have high threshold and relatively inefficient rejection capability. An example of discrimination information transfer on the part of males is the use of tapping by males of *D. palustris* (Grossfield, 1972) and *D. simulans* (Spieth, 1966) in deciding not to continue with decapitated foreign females. A similar use of this sensory input can be seen in the use of tapping by males of the virilis group (Spieth, 1951).

These divergent inputs of sensory modalities constitute the extrinsic component of discrimination; the intrinsic component of discrimination can be considered the stimulus recognition pattern responsible for stereotyped behavior (Grossfield, 1970a) or the rigid filtering system responsible for integrating the information. Owing to the existence of discernible point of information transfer, the mode of recognition of specific stimuli appears to offer the best route for dissection of a behavior. The degree of environmental lability of any component depends on its own behavioral architecture as well as whether it is a quantitative (for example, a continuum of excitability) or a qualitative factor (recognition of a specific stimulus). A comparison of the various species studied with respect to decapitation allows an evaluation of the organization of some of these components of courtship behavior.

In a no choice situation, decapitated females of *D. simulans*, *D. pseudoobscura*, or *D. hydei* were not inseminated due to their ability to resist males or the fact that males ignored them (Spieth, 1966). Presenting the males with normal and decapitated females simultaneously did yield a few inseminations of decapitated females. The facilitation of male courtship of decapitated females by the presence of normal females has been discussed elsewhere (Grossfield, 1972).

Males of two of the species studied here, however, do not inseminate decapitated females even in the presence of normal females and in spite of prolonged courtship by the males. This reflects differences in the behavioral architecture of courtship, since the failure to inseminate stems from disparate causes in the two sets of examples. In the species cited in Spieth's observations, the reasons revolved about the nature of visual information in the initiation of courtships while the present study suggests causal factors involving stimuli within the courtship sequence itself.

An example of one kind of behavioral architecture is the fact that no decapitated females of *D. guttifera* are inseminated. Males of this species presumably must see the wing spreading response before they attempt to mount (Grossfield, 1966). Since these females cannot give the response, no inseminations result. This illustrates a pattern where performance of a particular action constitutes a gating point or sign stimulus which cannot be bypassed by any other combination of stimuli (Grossfield, 1968, 1970a).

In contrast to this, *D. melanogaster* males do inseminate decapitated

females in a no choice situation and inseminate significantly more when normal females are also present (Spieth, 1966). *D. melanogaster* is a species capable of bypassing an acceptance response. Significantly, no single or multiple sensory deprivation experiment (Bastock & Manning, 1955; Bastock, 1956; Manning, 1959a; Grossfield, 1968) has succeeded in blocking courtship and copulation in this species; males always receive sufficient stimuli to eventually inseminate a significant proportion of females. Apparently any of a number of different kinds of stimuli are sufficient, as opposed to courtship patterns that at some point are locked-in on a particular stimulus as is that of *D. guttifera*. The organization of *D. melanogaster* behavior consists of a number of internally linked centers each with a fluctuating threshold and linked to the different courtship elements (Bastock & Manning, 1955). In addition, individuals are capable of accumulating a quantity of stimulation until threshold drops (Manning, 1959b). The "rape" situations that Spieth (1966) found with decapitated females of some species may be interpreted in the light of this capability. Stroking in the quinaria group provides an example of an autocatalytic activity capable of lowering thresholds. *D. melanogaster* has a flexible system, permitting wide variance in courtship sequence from one male to the next, and allowing a single organism to respond to a wide variety of courtship situations. Brown (1964) has stated that the *D. melanogaster* type of organization does not hold for *D. pseudoobscura*. The quinaria group species represent another departure from the *D. melanogaster* pattern.

The existence of sign stimuli in the courtship of certain light dependent species suggests that releasing mechanisms must exist as well, and these as discrete points of information transfer might be more amenable to genetic analysis. This is supported by the finding that the ability to mate in darkness appears to be at least under partial genetic control (Grossfield, 1966, 1970b).

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